Appendix 1 (as supplied by the authors): Detailed methods for study of enteric absorption and pharmacokinetics of oseltamivir in critically ill patients with pandemic (H1N1) Influenza

Methods

This was a multicentre, open-label pharmacokinetic (PK) study of adult (≥ 18 years age) patients admitted to 9 mixed community and academic intensive care units located in 3 cities in Canada (Winnipeg and Ottawa) and Spain (Tarragona). Subjects were required to have clinically suspected or laboratory-confirmed (positive polymerase chain reaction [PCR] of respiratory sections) pandemic (H1N1) influenza associated with severe respiratory failure (defined as requiring ventilator support) with new pulmonary infiltrates. The protocol received approval from the University of Manitoba Health Ethics Board and the research ethics boards of each participating institution. Informed consent was obtained from each patient or their substitute decision-maker.

All patients were initially prescribed oseltamivir (Tamiflu®, Roche, Basel, Switzerland) at a dose determined by their attending physician based on their clinical judgment. In most cases, the standard adult dose of 75 mg twice daily was utilized. Occasionally based on clinical judgment, oseltamivir was administered at double the standard treatment dose (i.e. 150 mg twice daily).

For each 75–150 mg dose, the powder from the capsule contents was dissolved in 5–20 mL of room temperature sterile water and injected down the nasogastric (NG) or nasojejunal (NJ) tube, followed by a flush with tap water. Standard supportive treatment was provided as clinically indicated. Clinical and physiologic data were was collected on each patient at admission and on the day that oseltamivir levels were drawn.

For the PK analysis, blood sampling was performed after receipt of at least the 4^{th} dose or higher, with baseline (predose), 2 h, 4 h, 6h, 9h, and 12h post dose samples. Four milliliters of blood were collected within vacutainers (Institution specific suppliers) containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. Plasma was separated by centrifuging at 2000 × g for 10 minutes and stored at –80°C until analysis. Coded samples were transported on dry ice to Bioanalytical Systems Inc. (BASi) in London, England, for assay of oseltamivir concentration. The analytical method was validated before use and based on previously published extraction and analysis parameters.[1] OS and OC were isolated from the human plasma samples after the addition of trideuterated internal standards by solid phase extraction. High performance liquid chromatography (HPLC) was performed using a Tomtec Quadra 96® Model 320 and detection was by tandem mass spectrometry monitoring of positive ions produced in the TurboIonSpray source of the Sciex API 3000. Processing was completed using Sciex Analyst software version 1.4.1 with a linear $1/x^2$ regression. The lower limit of detection for OS and the OC metabolite was 1.0 and 10 μg/L respectively.

Standard compartmental PK modeling was performed to characterize oseltamivir pharmacokinetics after oral (oro/nasogastric) route of administration. This analysis was performed using the nonlinear least-squares fitting program, ADAPT-5[2] assuming a one-compartment open model with first-order absorption and elimination for complete OS to OC metabolic conversion. In order to account for the difference in molecular weight of OC in comparison to the free base, the dose of the commercial product was adjusted for individualized modeling by a factor of 0.91.

The overall assay error pattern for OS and OC were determined by fitting a polynomial to the plot of the assay standard deviations (SD) versus plasma concentrations (C), producing the following formulas:

Oseltamivir (free base)	$SD = 0.04 + 0.083 * C$
Oseltamivir carboxylate	$SD = 1.2 + 0.14*C - 0.00002*C^2$

The ADAPT fitting routine weighted each individual plasma concentration to the iterated modeled concentration by the reciprocal of the assay error variance (i.e. $1 / SD^2$). The PK parameters identified were the absorption rate constant from the gastrointestinal tract (k_a), metabolic conversion (k_{met}) from OS to OC (i.e. assuming complete conversion), elimination rate constant of OC (k_{el}), apparent volume of distribution of OS (V_{OS}) and apparent volume of distribution of OC (V_{OC}). The t ½ abs_{os} is the half-life of absorption across the gastrointestinal tract of oseltamivir free base and was calculated from t ½ abs_{os} = natural logarithm of 2 / Ka. The t ½ $meta_{os}$ is the half-life of metabolic carboxylation of oseltamivir free base and was calculated from t ½ $meta_{os}$ = natural logarithm of 2 / k_{met}. The t $½_{oc}$ is the half-life of elimination of the oseltamivir carboxylate and was calculated as t $½_{oc}$ = natural logarithm of 2 / K_{el}. Since the fraction of drug absorbed (F) after oral administration was unknown, apparent volumes of distribution and clearances were reported as V_{OC}/F, and CL_{OC}/F. Individual clearance values were identified as the product of the associated volume times the elimination rate parameter (e.g. $CL_{OC}/F = V_{OC}/F * k_{el}$). Time to peak plasma concentration of oseltamivir (T_{max}) was calculated from $T_{max} = \ln((k_a/k_{met})*(1 / (k_a-k_{met}))$. Area under the concentration-time curves (AUC) representing a single dosing interval area at steady state was identified by dividing dose by CL/F, where the dose was adjusted for the molecular weight ratio with oseltamivir carboxylate. For examination of the OC elimination rate parameter, k_{el} as a covariate of renal function; the normalized Cockcroft-Gault equation was utilized as a marker of creatinine clearance, where Cl_{cr} = [mL/min/1.73m^2] = (140 - Age)* 88.4 / plasma creatinine [µmol/L], multiplied by 85% for females.[3] We also examined the role of Cockcroft–Gault equation using actual body weight (ABW) where, Cl_{cr} = (140 – Age)* 1.2* ABW / plasma creatinine [µmol/L], as well as ideal body weight (IBW) where, Cl_{cr} = (140 – Age)* 1.2* IBW / plasma creatinine [µmol/L] on correlating with OC elimination rate.

Pharmacokinetic parameters were non-normally distributed and thus reported as medians with their respective 25 to 75 percentile interquartile range. Demographic data were normally distributed and reported as means plus standard deviation. Because of the potential influence on a number of pharmacokinetic parameters with extracorporeal drug removal, the dialysis population was analyzed separately. Dose-normalization of PK parameters and concentrations was performed to allow for comparative inclusion of those patients who had received the non-standard regimen of 150 mg twice daily, an approach previously validated by Wattanagoon et al.[4] Clinical outcome parameters, such as duration of hospitalization and survival, were compared using actual values for drug concentration and area under the curve (AUC) for whichever regimen the subject was receiving. The nonparametric Mann-Whitney U-test was used for comparing the pharmacokinetic indices; the Student t-test was used for comparing parametric data, such as patient age, and body weight, between study cohort, and those dialysis patients requiring continuous renal replacement therapy (CRRT). All statistical tests used a two-tailed p-value of less than 0.05 as the limit to declare a difference between groups. SYSTAT version 11.00.01 (Chicago, IL) was use for all statistical analysis.

Appendix to Ariano RE, Sitar DS, Zelenitsky SA, et al. Enteric absorption and pharmacokinetics of oseltamivir in critically ill patients with pandemic (H1N1) influenza. *CMAJ* 2010. DOI:10.1503/cmaj.092127.

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